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# Structure and Function of the Reovirus Genome

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## INTRODUCTION

Reoviruses possess several unique properties: their genome consists of double-stranded (ds) ribonucleic acid (RNA) and exists in the form of 10 individual genes that are not linked covalently, and the reovirus particle, which consists of two concentric icosahedral capsid shells, contains a ds → single-stranded (ss) RNA transcriptase that transcribes the ds genome into messenger RNA (mRNA).

There are three serotypes of mammalian reovirus, 1, 2 and 3, which are ubiquitous. There are also several serotypes of avian reoviruses, antigenically unrelated to the mammalian ones. In addition, there are numerous other viruses of vertebrates, insects, and plants that share the unique properties listed above, but differ in the number of genes that they possess and in details of virus particle structure. These viruses are grouped together into the family Reoviridae (Table 1). All appear to use the same strategy of genome expression.

This review is concerned with the mammalian orthoreoviruses. I will attempt to trace the highlights of research on reovirus, demonstrating how one discovery or concept led to the next, and focus on those unique aspects of reovirology that have stimulated research on and provided insight into other areas of virology and cell biology.

## DISCOVERY OF REOVIRUSES

Reoviruses were discovered in the early 1950s, when tissue culture methods permissive of virus growth in vitro were being developed and when, under the influence of efforts to develop mass vaccination against poliomyelitis, interest in enteric viruses was intense. Beginning in 1950, many cytopathogenic agents that were neither poliovirus nor coxsackievirus were encountered in the human alimentary tract (77, 79). These agents, many of which were isolated from apparently healthy persons, turned out to possess rather similar properties, and they were lumped together under the acronym ECHO virus (enteric cytopathogenic human orphan). Sometimes referred to as viruses in search of diseases, many of them are in fact associated with clinical disease. They are now known to be members of the *Enterovirus* genus of the Picornaviridae family. Some isolates, however, although meeting the original and appropriately loose definition of ECHO viruses, were soon found to be different; the best known of these are the reoviruses. ECHO virus 10, in particular, was recognized as being much bigger and producing distinctive cytopathic effects (77), and it became reovirus serotype 1 (82). Soon two viruses previously isolated from macaca monkeys and designated SV12 and SV59 were shown to be identical to reovirus serotypes 1 and 2, respectively (80), and a virus originally isolated from humans in suckling mice (107) and found to produce hepato-encephalomyelitis in mice turned out to be the reovirus that had been designated serotype 3 (106).

TABLE 1. *The Reoviridae*

| Genus | Virus | Host | Symptoms in humans | No. of genes |
|---|---|---|---|---|
| *Orthoreovirus* | Mammalian reoviruses (3 serotypes) | Humans and other mammals | None | 10 |
| | Avian reoviruses | Birds | | |
| *Orbivirus* | Numerous isolates; transmitted by insects | Mammals and insects (culicoides, mosquitoes, ticks) | Encephalitis (rare) | 10 |
| | Bluetongue virus | | | |
| | African horsesickness virus | | | |
| | Eubenangee virus | | | |
| | Colorado tick fever virus | Humans, ticks | Encephalitis | 12 |
| *Rotavirus* | Human rotavirus (2 serotypes) | Humans | Infantile gastroenteritis (diarrhea) | 11 |
| | Calf rotavirus (Nebraska calf diarrhea virus) | Calves | | |
| | Murine rotavirus (epizootic diarrhea of infant mice [EDIM]) | Mice | | |
| | Simian rotavirus (SA11) | Monkeys | | |
| | Bovine or ovine rotavirus ("O" agent) | Cattle or sheep | | |
| | Numerous other rotaviruses | Other mammalian species | | |
| *Cypovirus* | Cytoplasmic polyhedrosis viruses (numerous strains) | *Bombyx mori* (silkworm) and other *Lepidoptera*, *Diptera*, and *Hymenoptera* | | 10 |
| *Phytoreovirus* | Wound tumor virus | Plants, leaf hoppers | | 12 |
| | Rice dwarf virus | Plants, leaf hoppers | | |
| *Fijivirus* | Maize rough dwarf virus | Plants, leaf hoppers | | 10 |
| | Fiji disease virus | Plants, leaf hoppers | | |

## STRUCTURE OF THE REOVIRUS PARTICLE

The broad outlines of reovirus structure were laid in the early 1960s. Starting with Rhim et al. (78), a long series of electron microscopic studies led to the following conclusions. The reovirus particle consists of two capsid shells with diameters of about 50 and 75 nm, respectively (Fig. 1). Both consist of subunits or capsomers arranged with icosahedral symmetry, but their precise arrangement has been very difficult to determine and is, in fact, still not resolved. Whereas originally an outer capsid shell composed of 92 columnar capsomers or 180 truncated pyramidal capsomers arranged around 92 holes was suggested (111), more recently Luftig et al.(57) have proposed that there are 122 capsomers, based on the observation that 20 capsomers are clearly visible at the periphery of negatively stained virus particles. More recently, Palmer and Martin (72) have suggested that there are 32 morphological units arranged with T = 3 symmetry, each of which has the symmetry of a $T = 9$ icosadeltahedron, being made up of six separate wedge-shaped subunits that are in turn made up of three smaller subunits. A prominent feature of this structure is the sharing of subunits, an apparently unique feature of all members of the Reoviridae that makes precise assignment of the symmetry system very difficult.

The outer capsid shell of reovirus is readily digested by proteases like trypsin and chymotrypsin, thereby yielding single-shelled cores. The arrangement of capsomers in the core is even less defined than that in the outer capsid shell. Cores possess a unique structural feature: they exhibit 12 prominent icosahedrally located projections or spikes that are hollow, thus providing means of access to and exit from the particle interior (57).

## REOVIRUS GENOME

The studies of Gomatos and collaborators in the early 1960s that indicated that the RNA of reovirus particles was double stranded created a great deal of interest, since they provided the first demonstration of the occurrence of ds RNA in nature. The original observation was that cytoplasmic inclusions in L cells infected with

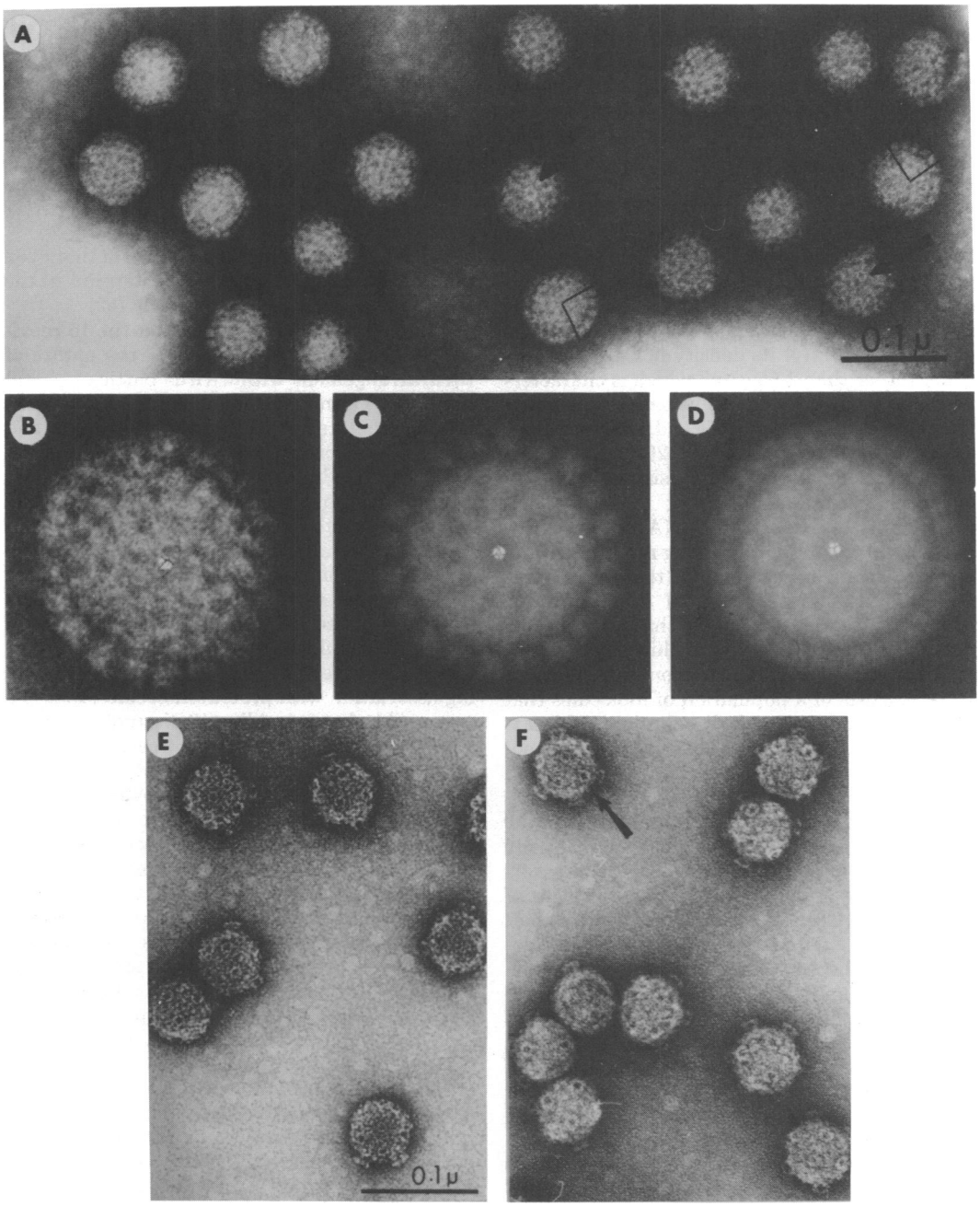


FIG. 1. *Reovirus particles and reovirus cores. (A) Reovirus particles negatively stained with phosphotungstic acid. Arrows point to particularly clear capsomer-like structures. (B) A reovirus particle photographed after 10-fold (C) and 12-fold (D) rotational enhancement. Note how structural details are brought out by the former; in particular, the number of peripheral capsomers is clearly seen to be 20, rather than a multiple of 6. See Luftig et al. (57) for a discussion of the significance of this observation for constructing models for reovirus structure. (E and F) Reovirus cores stained with phosphotungstic acid. Note the icosahedral arrangement of the projections/spikes (57).*

reovirus fluoresced pale green when treated with acridine orange (27). This is indicative of ds nucleic acids which bind only a small amount of the dye and therefore stain orthochromatically; ss nucleic acids, which bind more dye, stain metachromatically and fluoresce bright red. The

inclusions proved to be resistant to deoxyribonuclease but susceptible to ribonuclease (RNase) at low salt concentrations, and it was soon shown that the nucleic acid isolated from purified virus particles was indeed ds RNA. The evidence rests on the following facts, among others: (i) the RNA exhibits very sharp melting profiles, with the $T_m$ depending on the ionic strength (9, 88); (ii) it is resistant to RNase, the resistance depending on the concentration of both monovalent and divalent cations, as well as on the concentration of RNase (9, 88); (iii) it is susceptible to RNase III, which is specific for ds RNA; (iv) formaldehyde fails to induce hyperchromicity (26); (v) its density in $Cs_2SO_4$ is 1.61 g/ml, rather than 1.65 g/ml, which is characteristic of ss RNA (33, 88); (vi) its base composition indicates equality of adenine and uridine, as well as of guanine and cytosine (9, 26); and (vii) X-ray diffraction patterns are consistent with double strandedness (3, 49).

Attempts to extract this RNA from reovirus particles in the form of one long molecule failed, and evidence soon accumulated that the reovirus genome exists in the form of a collection of discrete and unique segments that proved to be genes. The first indication of this was the electron microscopic demonstration that reovirus RNA consists of a population of molecules that exhibit a trimodal length distribution with maxima at 1.1, 0.6, and 0.35 μm, corresponding molecular weights of about 2.5, 1.4, and 0.8 million (16, 110). Clearly, even the largest of these molecules corresponded to only a portion of the genome, since reovirus particles had already been shown to contain about 14.6% RNA, corresponding to an aggregate molecular weight of at least $10 \times 10^6$ (26). It was then shown that, regardless of the means used to liberate it, reovirus RNA displays three size classes of molecules when analyzed on sucrose density gradients: these are the L, M, and S species of molecules, which sediment with 14, 12, and 10.5S, corresponding to molecular weights of about 2.7, 1.4, and 0.7 million, respectively, or about 4,500, 2,300, and 1,200 nucleotide base pairs (9, 88, 112). The molecules in these three size classes were shown to be discrete segments rather than random fragments of larger molecules by the fact that they did not hybridize with each other and that they were transcribed into specific species of mRNA molecules within infected cells (7, 9, 114). These three size classes were then further separated by polyacrylamide gel electrophoresis into 10 discrete and unique molecular species (Fig. 2) which are present in equimolar amounts and possess an aggregate molecular weight of about $15 \times 10^6$ (93). Final proof of the segmented nature of the reovirus

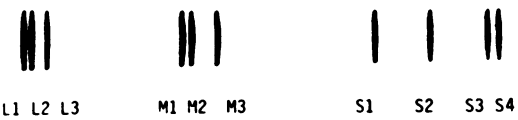


FIG. 2. *Autoradiogram of a gel in which the 10 genes of the genome of reovirus serotype 3 (strain Dearing) had been electrophoresed. The direction of electrophoresis was from left to right. (Courtesy of A. R. Schuerch.)*

genome came with the demonstration that even before extraction there are 20 3′ termini in the RNA within each reovirus particle (62).

Although there is no doubt that the 10 reovirus genes are discrete molecules, the nature of their arrangement within virus particles is still not clear. Granboulan and Niveleau (28) were able to liberate, with very low frequency and demonstrable only by electron microscopic analysis, very long linear arrays of RNA from reovirus particles, and more recently Kavenoff et al. (40) were able to release the RNA in the form of spider-like arrangements, with all genes connected at a common focus. These authors hypothesize that within the virus particles the 10 genes are linked noncovalently by protein molecules. It should be noted in this regard that some highly specific mechanism, possibly involving association with protein molecules, must be responsible for assembling 10 discrete species of RNA into each virus particle (see below). This mechanism must be very efficient, since under optimal conditions the ratio of reovirus particles to infectious units has been reported to approach unity (105). The pattern of transcription of individual genes within reovirus cores, however, is more in accord with the view that the individual genes are unlinked, for they are transcribed not with equal frequencies, but with frequencies that are inversely proportional to their size (99). This is impossible if they are linked in a linear array if the catalytic sites of the transcriptase are fixed in space (which is most probably the case; see below), unless they are arranged in decreasing order of transcription frequency, as are the genes of vesicular stomatitis virus. This is unlikely, since the relative transcription frequencies can be made to change significantly by varying the concentration of $Mg^{2+}$ and nucleoside triphosphates. Thus, the genes are probably not linked within the virus particle. Analysis by X-ray crystallography suggests well-ordered packaging of the genes with adjacent helices being packed parallel to one another (30).

## PROTEINS OF REOVIRUS

The protein composition of reovirus particles was first examined by Loh and Shatkin (56) and Smith et al. (102). The nomenclature of reovirus

proteins was developed by the latter group of workers.

Reovirus particles are composed of nine species of proteins (Table 2). The outer capsid shell is composed of three species: the major species μ1C (a cleavage product of μ1) and σ3, which together make up over 60% of the mass of the reovirus particle, and the minor species σ1, which is present in reovirus particles to the extent of only about 24 molecules. The core, which comprises about one-third of the reovirus particle's protein mass, contains six protein species: the major components λ1, λ2, and σ2, and the minor components λ3, μ1, and μ2. The structural, antigenic and enzymatic functions of these proteins, as far as presently known, are as follows.

(i) No function can yet be assigned to the minor protein species λ3, μ1, and μ2 (12 molecules or less per virus particle). All are present in cores, but their location is not known: they may be located on the inner surface of the core shell, or they may be associated with the viral genome, or they may occur free within the core. Conceivably, they may be responsible for some of the enzymatic activities exhibited by reovirus cores (see below), or they may serve to link the genes (see above).

(ii) Protein λ2 is the major, if not the sole, component of the 12 core projections/spikes (120) through which, as Gillies et al. showed in 1971 (24), the ss transcripts of reovirus genes that are synthesized in cores are liberated. As demonstrated via cross-linking studies (73), each spike is a pentamer of λ2, so that there are 60 λ2 molecules per virus particle. Recently Lee et al. (51) isolated and cloned a series of hybridomas that secrete immunoglobulin Gs (IgGs) directed against a variety of reovirus-coded proteins and found two that secrete IgG's directed against protein λ2. Interestingly, these IgG's also neutralize reovirus infectivity, prevent hemagglutination, and precipitate virus particles. This evidence indicates that, contrary to what had been believed, the projections/spikes project through the outer capsid shell to the surface of the virus particle (31). Models of reovirus architecture will thus have to take into account the fact that there are 12 unique icosahedrally distributed structural environments on the surface of reovirus particles. It is remarkable that they have not been seen.

TABLE 2. *Reovirus proteins*

| Protein species | Mol wt | % in virion | Approx no. of molecules/virus particle | Location |
|---|---|---|---|---|
| Structural | | | | |
| λ1 | 155,000 | 15 | 105 | Core |
| λ2 | 150,000 | 11 | 90 | Core |
| λ3 | 135,000 | ≤2 | ≤12 | Core |
| μ1 | 80,000 | 2 | 20 | Core |
| μ1C | 72,000 | 35 | 550 | Outer shell |
| μ2 | 70,000 | ≤2 | ≤12 | Core |
| σ1 | 42,000 | 1 | 24 | Outer shell |
| σ2 | 38,000 | 7 | 200 | Core |
| σ3 | 34,000 | 28 | 900 | Outer shell |
| Nonstructural | | | | |
| μNS | 75,000 | | | |
| σNS | 36,000 | | | |

(iii) Proteins λ1 and σ2 are the other two major core components. It is tempting to speculate that the core shell is composed of capsomers made up of 1*n* λ1 and 2*n* σ2 molecules (just like the outer reovirus capsid shell, which may consist of capsomers made up of 1*n* μ1C and 2*n* σ3 molecules; see below). Since protein λ1 is more readily iodinated than σ2 (120), it may be closer to the core surface (assuming that both proteins can be iodinated to similar degrees, other factors being equal, which has not yet been shown).

(iv) Of proteins μ1C and σ3, protein μ1C is a cleavage product of protein μ1. It is the principal component of the reovirus outer capsid shell, as well as of reovirus particles. Antibodies against it do not neutralize infectivity or prevent hemagglutination, although they may precipitate virus particles (31).

Protein σ3 is the other principal constituent of the outer capsid shell. It possesses strong affinity for μ1C, as is shown by the fact that well over half of the unassembled form of both of these two proteins exists in the cytoplasm of infected cells complexed with each other (32, 51). Presumably, therefore, these proteins are also intimately associated with each other in virus particles, in which they may exist in the form of capsomers with the constitution 1*n* μ1C:2*n* σ3. It should be noted, however, that (i) chymotrypsin removes σ3 from reovirus particles before μ1C is degraded (37), (ii) after infection, reovirus particles are converted to subviral particles from which σ3 is removed completely, whereas μ1C loses only a polypeptide with a molecular weight of about 12,000, being converted to protein δ (13, 96, 98), and (iii) antibody to σ3 neutralizes infectivity and possesses hemagglutination inhibition activity, whereas antibodies to μ1C do not (51). Thus, although, closely associated, protein σ3 and μ1C can also react independently.

Protein σ3 possesses the remarkable property of having strong affinity for ds RNA; also, that portion of it that occurs in free form (that is, not complexed with μ1C) in the cytoplasm of infected cells can be isolated by adsorption to and elution from polyinosinic-polycytidylic acid (32).

The significance of this property, a remarkable one for a protein that is a component of the outer capsid shell, is not known. Perhaps σ3 has some function during reovirus morphogenesis.

(v) Protein σ1 is only a minor component of reovirus particles (only 24 molecules), but it is very important, since it specifies how reovirus particles interact with host cells and with the host. Antibody to λ2, but not antibodies to μ1/μ1C or σ3, prevents antibody against σ1 from binding to σ1; this indicates that σ1 is located close to where the projections/spikes penetrate through the outer capsid shell to the outer particle surface (52). Presumably two σ1 molecules are associated with each of these structures. Protein σ1, which is able to adsorb to cells by itself, is the reovirus cell attachment protein (52). It is also the reovirus hemagglutinin (119) and elicits the formation of neutralizing antibody (116). It is responsible for the development of delayed hypersensitivity (117) and for the generation of suppressor T cells (19) and of cytolytic T lymphocytes (18). It also determines the extent to which reovirus particles interact with microtubules (5) and specifies reovirus tissue tropism and virulence (115) (see below). It is by far the most type specific of all reovirus proteins: the ability of antibodies against it to precipitate homologous and heterologous σ1 molecules (that is, those of serotypes 3 and of 2 and 1, respectively), to neutralize virus, and to inhibit hemagglutination is almost completely type specific (23, 31, 51). By contrast, antibodies against λ2 and σ3 display hemagglutination inhibition activity that is mostly type specific, protein-precipitating ability that is partially type specific, and neutralizing ability that is group specific (31, 51). It seems that the antibodies against λ2 are mainly responsible for the group-specific neutralizing elements in antisera against reovirus, and that the antibodies against σ1 are responsible for the type specificity of the neutralizing activity of such antisera.

## INFORMATION CONTENT OF THE 10 REOVIRUS GENES

By the late 1960s and early 1970s, it had been established that the three size classes of reovirus genes, namely, the *L*, *M*, and *S* genes (6), code for the three size classes of reovirus proteins, namely, the λ, μ, and σ size class proteins (102). It was realized, however, that the largest *L* gene did not necessarily code for the largest λ protein, and so on. Techniques for identifying the information content of individual genes became available in the late 1970s. They came from two quite different directions, but yielded identical answers. The first involved translation in an in vitro protein-synthesizing system. Attempts to translate reovirus mRNA's in vitro had been made since the early 1970s, when McDowell and Joklik (60) demonstrated that polyribosomes isolated from infected cells were capable of incorporating labeled amino acids into proteins with the electrophoretic mobilities of authentic reovirus proteins. Shortly thereafter, McDowell et al. (61) devised cell-free systems from several mammalian cells, including HeLa cells, L cells, and Ehrlich ascites tumor cells, that were capable of translating reovirus mRNA species transcribed in vitro by reovirus cores into σ, μ, and even λ size class proteins, and Both et al. (12) devised a similar system from wheat germ that was capable of translating all 10 species of reovirus mRNA. In fact, it was in this study that the existence of the two minor protein species λ3 and μ2 was first demonstrated (not only are these two proteins present in virus particles and in extracts of infected cells in very small amounts only, but their electrophoretic migration rates are also very close to those of the major capsid protein species λ2 and μ1C, so that they are usually obscured by them). The straightforward way of determining the information content of reovirus genes by this approach would be to isolate the 10 species of mRNA transcribed by reovirus cores in vitro, to hybridize them to the various genes to determine from which each was transcribed, and to translate them individually in an in vitro protein-synthesizing system. It is difficult technically, however, to isolate sufficient quantities of the larger mRNA species. The approach therefore adopted by McCrae and Joklik (59) was to isolate the 10 genes themselves, to denature them at 50°C in 90% dimethyl sulfoxide, and then to dilute them into a protein-synthesizing system prepared from wheat germ. At the RNA concentration and ionic strength used in these incubated mixtures, reannealing of the plus and minus strands was sufficiently slow to permit the translation of even *l* size class mRNA molecules into complete protein molecules. Identification of proteins was achieved not only by comparison of the electrophoretic mobilities of the translation products with those of authentic proteins, but also by V8 peptide mapping. It was found that there was no absolute correspondence between relative gene and protein size as judged by electrophoretic migration rates. The gene-protein assignments that were found are summarized in Fig. 3.

Mustoe et al. (67) studied the same problem by a completely different method. As described above, there are three serotypes of mammalian reovirus, serotypes 1, 2, and 3. The sizes of the genes of virus strains belonging to these three serotypes, and of the proteins encoded by them, differ slightly but detectably. As would be ex-

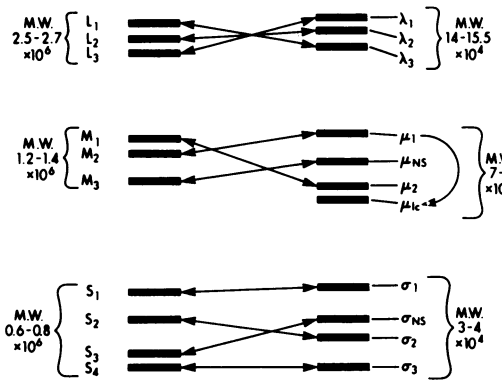


FIG. 3. *Gene-protein assignments for reovirus serotype 3 (strain Dearing) (59).*

pected, the genes of these three serotypes undergo extensive reassortment in mixedly infected cells. Thus, by examining polyacrylamide gel electrophoresis profiles of the RNA and protein species of cloned recombinants after pairwise mixed infection, it is possible to determine which protein changes accompany specific gene changes. The gene-protein assignments made by this method are exactly the same as those yielded by the direct in vitro translation method described above.

## ENZYMES IN REOVIRUS

In 1967, Kates and McAuslan (39) discovered the first synthetic virus-coded virus-associated enzyme, deoxyribonucleic acid (DNA)-dependent RNA polymerase of vaccinia virus. A year later, Borsa and Graham (10) and Shatkin and Sipe (91, 92) independently discovered an enzyme with a similar function, namely, to transcribe the viral genome into mRNA, in reovirus particles or, more acurately, in reovirus cores: this is a ds → ss RNA polymerase, or "transcriptase." Shortly thereafter, Kapuler et al. (38) and Borsa et al. (11) demonstrated the presence of nucleoside triphosphate phosphohydrolase activity in reovirus cores, and in 1975 Furuichi and Shatkin and their collaborators discovered that terminal guanylyltransferase and the enzymes that methylate the cap G and the ribose of the original 5'-terminal residue are also present (21, 89). Together these enzymes constitute a mechanism that enables reovirus cores to transcribe the 10 viral genes into 10 capped species of mRNA which are extruded through the 12 projections/spikes located on cores (24).

The five mRNA-synthesizing enzyme activities are not expressed by intact reovirus particles: they are expressed only when their outer capsid shell is partially or completely removed. Two types of particles in which these enzymes are expressed are the subviral particles (SVP), which are formed in infected cells and have lost all outer shell proteins except a 60,000-dalton portion of protein μ1C (see below), and the reovirus cores, which have lost all outer capsid shell. Presumably, loss of integrity of the outer capsid shell causes structural changes in the core as a result of which the transcriptase and the capping enzymes are activated. Interestingly, the process is reversible, for addition of σ3 to SVP causes the enzymes to become latent (4). Nothing is known concerning the nature of the changes that are induced in cores as a result of which they become able to transcribe. One possibility is that the λ2 clusters that make up the projections/spikes are closed in virus particles and open in cores; alternatively, the crucial factor may not be the configuration of the spikes, but rather changes in the configuration of the transcriptase and associated enzymes which may be locked into an inactive state if the outer capsid shell is intact.

Nothing is known concerning the nature of the transcriptase or of the four enzymes concerned with capping transcripts, since all enzyme activity is lost as soon as cores are disrupted. It is therefore thought that the enzymes are components of the core shell and that the genes move past transcriptase catalytic sites fixed on the interior side of the core shell, perhaps at the base of the spikes, so that the ds templates remain within the cores, whereas the transcripts are fed into and through the spikes. If this is correct, the transcriptase catalytic site may comprise sequences on both λ2 and on neighboring protein molecules (λ1 or σ2), whereas the capping functions would be on λ2 in the projection/spike channels. Indeed, when cores transcribe ds RNA in the presence of labeled pyridoxal phosphate, which is hypothesized to react with the active site of the enzyme, both λ1 and λ2 become labeled (63). It should be pointed out, however, that this is the only evidence that the transcriptase is located on the inner capsid shell. As discussed above, no functions have yet been assigned to the three major proteins, λ3, μ1, and μ2, that are also associated with cores, and these proteins, either free in the interior of the virus particle or attached to the inner core shell, may themselves possess transcriptase or capping activity or both.

## TRANSCRIPTION OF REOVIRUS RIBONUCLEIC ACID

The transcriptase activity of reovirus cores is very stable; the enzyme is active for over 48 h at 48°C, which is its optimal temperature. The transcription that it catalyzes is totally asymmetric—strands with only one polarity (plus, that is, translatable) being synthesized—and conservative (that is, the first plus strand to be formed is already a transcript, not the plus

strand of the ds molecule being transcribed). In the presence of optimal $Mg^{2+}$ concentrations, all genes are transcribed at the same rate, i.e., four and two times as many *s* and *m* class transcripts, respectively, are synthesized as *l* class transcripts (99). In other words, the frequency of transcription initiation is not the same for each gene, but is inversely proportional to gene size, which argues against the 10 genes being transcribed as a linked complex (see arguments above).

The transcripts that are formed during the early part of the reovirus multiplication cycle or in vitro in the presence of *S*-adenosylmethionine are capped and methylated; that is, the structure of their 5′ termini is $m^7G(5')ppp(5')G^mpCp$ ... (21, 89). However, neither capping nor methylation is tightly coupled to transcription. *S*-adenosylmethionine does not stimulate transcription, and under appropriate conditions not only unmethylated but also uncapped transcripts are readily formed (22). Interestingly, *S*-adenosylmethionine stimulates the transcriptase of orbiviruses 2-fold (109) and that of cytoplasmic polyhedrosis viruses by up to 70-fold (20). The mechanism of this stimulation is thought to be lowering of the $K_m$ of the initiating nucleotide at the promoter site.

Although not influenced by *S*-adenosylmethionine, the initiation of transcription of reovirus genes appears to be a complex process, for Yamakawa et al. (M. Yamakawa, Y. Furuichi, K. Nakashima, A. J. LaFiandra, and A. J. Shatkin, J. Biol. Chem., in press) have recently found that transcription aborts in more than 50% of initiation events before transcripts are more than five residues long. This finding relates to a series of observations made about 10 years ago to the effect that reovirus particles contain not only ds RNA, but also ss RNA. In fact, about 25% of the total RNA in reovirus particles is ss RNA (6, 92). This RNA is in the form of short molecules which are not breakdown products since they contain PPP (as well as smaller amounts of PP and P) at their 5′ ends. These molecules fall into two classes (8, 69, 108). About one-third contain only adenosine; these are the oligoadenylates, and there are about 900 of them in each reovirus particle. The remainder fall into the following series: $GC_{OH}$; $GCU_{OH}$; $GCUA_{OH}$; $GCUA(U)_{1-4}U_{OH}$; and $GCUA(A)_{1-3}A_{OH}$: these are the 5′-G-terminated oligonucleotides. The sequence of these 5′-G-terminated oligonucleotides is very interesting, since all reovirus plus strands share the sequence GCUA at their 5′ termini (41, 54; J. K.-K. Li, J. D. Keene, P. P. Scheible, R. Chmelo, J. Antzak, and W. K. Joklik, unpublished data). They are therefore clearly products of abortive transcription. The fact that they are sealed into reovirus particles lends support to the view (see above) that the projections/spikes through which transcripts are normally released are sealed in reovirus particles and open in reovirus cores. (Incidentally, this hypothesis also implies that nucleoside triphosphates are unable to enter intact reovirus particles, thereby rendering transcription impossible, irrespective of whether the transcriptase in virus particles exists in a configurationally active or inactive state). Presumably these oligonucleotides are sealed into reovirus particles during the final stage of morphogenesis, when transcription becomes inhibited through the association of σ3 to the penultimate form of immature virus particles (see above). Either the 5′-G-terminated oligonucleotides may then represent the normally occurring abortive transcripts demonstrated by Yamakawa et al. (in press) which become trapped, or transcription elongation may at that time be inhibited before transcription initiation, the abortion rate then rising briefly to 100% (8). Furthermore, the synthesis of oligoadenylates may then be an expression of a partial activity of the transcriptase in the process of being inactivated: its $K_m$ for adenosine triphosphate may then greatly exceed that for other nucleoside triphosphates, which would result in its catalyzing the template-independent polymerization of adenosine triphosphate to short oligoadenylates. Indeed, Silverstein et al. (95) found that oligoadenylates are formed within nascent virus particles during the final stages of morphogenesis and suggested that the oligoadenylate polymerase activity may be an alternative activity of the viral transcriptase regulated by outer capsid proteins. Furthermore, Johnson et al. (36) found that late temperature-sensitive mutants of reovirus that synthesize noninfectious and therefore presumably defective particles at nonpermissive temperatures do not synthesize oligoadenylates at nonpermissive temperatures, which again supports the conclusion that they are synthesized during the final stages of virus maturation.

## SEQUENCES OF REOVIRUS GENES AND MESSENGER RIBONUCLEIC ACIDS

Attempts to sequence reovirus genes started soon after it was realized that they were individual RNA molecules, the purpose being to test the possibility that the association of sets of genes during morphogenesis involved recognition of complementary sequences at their ends. Although at that time, in the early 1970s, methods were available for examining only the ter-

minal two or three residues, it was soon shown that all reovirus genes had identical terminal dinucleotide pairs, which ruled out the possibility of terminal complementarity. The first serious attempt to sequence a reovirus RNA molecule was when Nichols et al. (70) found that under certain limiting conditions reovirus cores synthesize only a single species of mRNA, the *s4* species (15), and sequenced its first 25 5′-terminal residues. This work was followed by the studies of Kozak and Shatkin, who, in a series of elegant studies, sequenced the regions in 6 of the 10 species of mRNA that bound to and were protected from digestion with RNase by wheat germ 40S ribosomal subunits and 80S ribosomes (41, 44–46). In all cases the 40S subunits protected sequences 50 to 60 residues long that included the 5′ terminus as well as the initiation codon. The sequences protected by intact 80S ribosomes were subsets of these sequences that were only about one-half as long and were centered around the initiation codons (41). Figure 4 shows the six sequences. Interesting features are: (i) all six 5′ termini start with GCUA; (ii) the distance between the 5′ terminus and the first initiation codon is short, only 15 to 33 residues; (iii) the sequences between the 5′ termini and the initiation codons are completely different; (iv) these sequences lack secondary structure features such as hairpins; and (v) each mRNA possesses near its 5′ terminus a Shine and Dalgarno (94) sequence, that is, a sequence that is complementary to some sequence near the 3′ end of the 18S ribosomal RNA.

Recently Li et al. (54) sequenced the 3′ ends of both strands of the *S*1 genes of serotypes 1, 2, and 3. Since the plus and minus strands of reovirus genes are exactly the same length and the plus strands of reovirus genes are identical with reovirus mRNA molecules (55, 68), this analysis provided the sequences at both ends of the *s*1 mRNA molecules of the three reovirus serotypes. As pointed out above, the *S*1 gene codes for protein σ1, the most type specific of all reovirus proteins. Examination of the sequences of the three *S*1 genes should therefore reveal most clearly those features that are conserved and those that may be varied. In fact, one would expect few similarities among their coding sequences, but significant similarities in their terminal sequences, which should be able to recognize RNA polymerases, ribosomes, encapsidation signals, and, perhaps, sequences on other reovirus genes. Li et al. (54) did, in fact, find that all three *S*1 genes possess at least six and in some cases nine identical base pairs at the ends that contain the 5′ ends of the plus strains (Fig. 5); that the initiation codons are 14 or 15 base pairs downstream and that the sequences between the common terminal ones and the initiation codons, for even these three homologous genes, are completely different; that the three coding sequences are quite different (although the ratios of neutral to charged to hydrophobic amino acids are similar); and that at the other end of the genes there is substantial homology, including a terminal block of five base pairs and three other blocks of homology from five to seven base pairs long separated by regions of varying length. Similar studies on the three *L*3, *M*3, and *S*2 genes have shown that all possess the same four and five respectively terminal base pairs that are also present on *S*1 genes; that the sequences between the 5′ termini of plus strands and the initiation codons of homologous genes are identical (rather than completely different, as in *S*1 genes); that the coding sequences of homologous genes are identical for the first 10 to 20 codons; and that at the other ends the *L*3, *M*3, and *S*2 genes of serotypes 1, 2, and 3 are

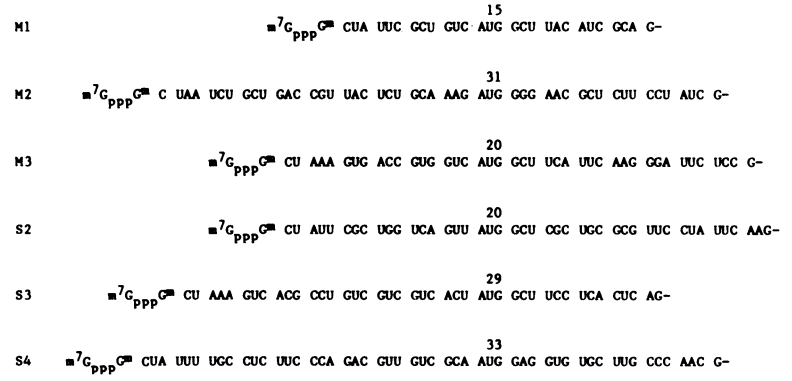


FIG. 4. *5′-Terminal sequences of six species of mRNA of reovirus serotype 3 (strain Dearing). The sequences shown are those protected by the 40S ribosomal subunits of wheat germ (41). The gene assignments are from Darzynkiewicz and Shatkin (15).*

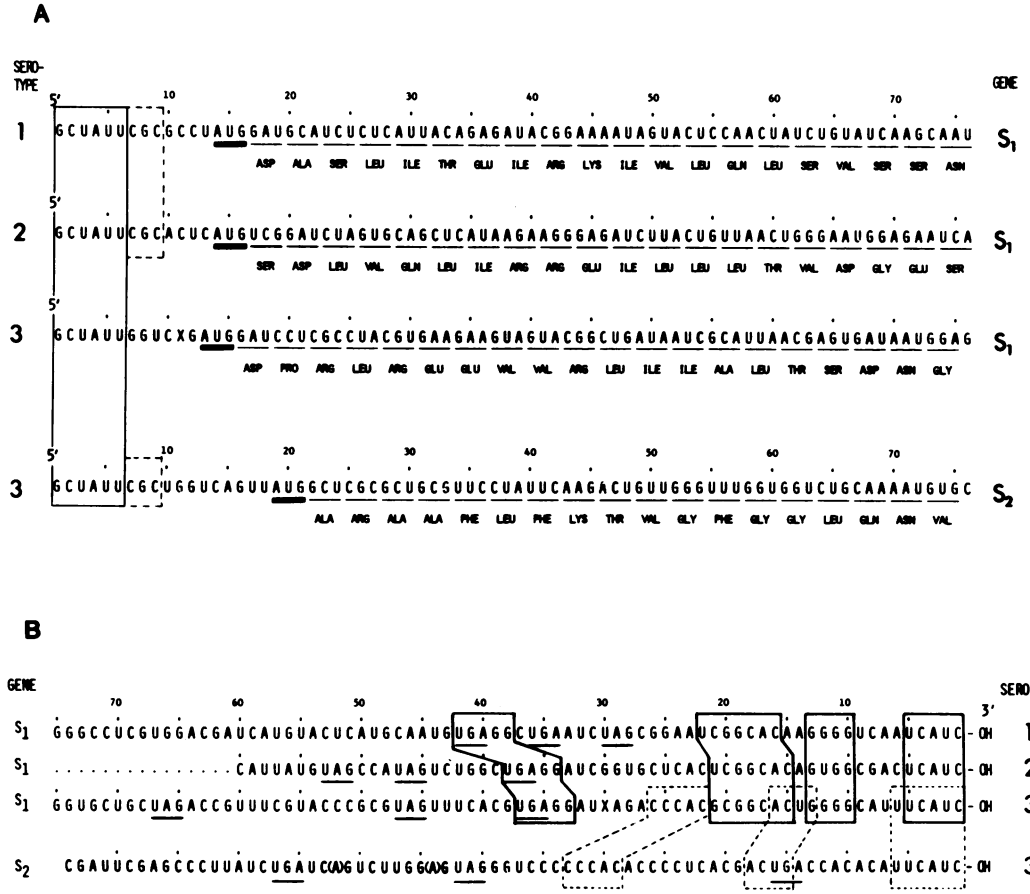


FIG. 5. *Sequences of the 5′ termini (A) and 3′ termini (B) of the plus strands, that is, the mRNA's, of the S1 genes of reovirus serotypes 1, 2, and 3 and of the S2 gene of reovirus serotype 3 (54).*

also much more similar than the three *S*1 genes (Joklik et al., unpublished data).

The possible secondary structure of reovirus mRNA's is interesting; Fig. 6 shows a typical example, the *s*1 mRNA of serotype 2 (54). There is little tendency to form hairpin loops within the terminal 70 to 100 residues at either end; the sole exception is a hairpin loop near the 3′ end that contains three in-phase termination codons. Some mRNA species such as the *s*2 mRNA of serotype 3, do not even possess this loop. There are complementary sequences 6 to 10 residues long near the 5′ and 3′ ends of all reovirus mRNA's that have been examined, the free energy of association of which is quite high (−10 to −20 kcal) (see also Li et al. [55]). There is also a six-residue-long sequence near the 5′ end of serotype 2 *s*1 mRNA that is complementary to a sequence near the 3′ end of 18S ribosomal RNA (a Shine and Dalgarno sequence) and that overlaps almost entirely the sequence that is complementary to the 3′-terminal sequences. Each reovirus mRNA species possesses a different Shine and Dalgarno sequence. For some RNA species, such as serotype 3 *s*1 or *s*3 mRNA, it is no more than four residues long and probably very unstable; for some RNA species, such as serotypes 3 *s*2 mRNA (55), the Shine and Dalgarno sequence does not overlap at all with the sequence that is complementary to the 3′-terminal sequence. Thus it seems that sequences immediately upstream from the initiation codon of reovirus mRNA's can associate either with sequences near their own 3′ terminus or with 3′-terminal 18S ribosomal RNA sequences. Conceivably, such associations could regulate the frequency of translation of the individual species of reovirus mRNA. As will be discussed below, some reovirus mRNA species are translated much more (up to 50-fold) frequently than others. The system of 10 species of reovirus mRNA molecules, which are all translated in the same environment at the same time and the relative concentrations of which within infected cells can be measured accurately by hybridization to ds RNA, is uniquely suited for elucidating the na-

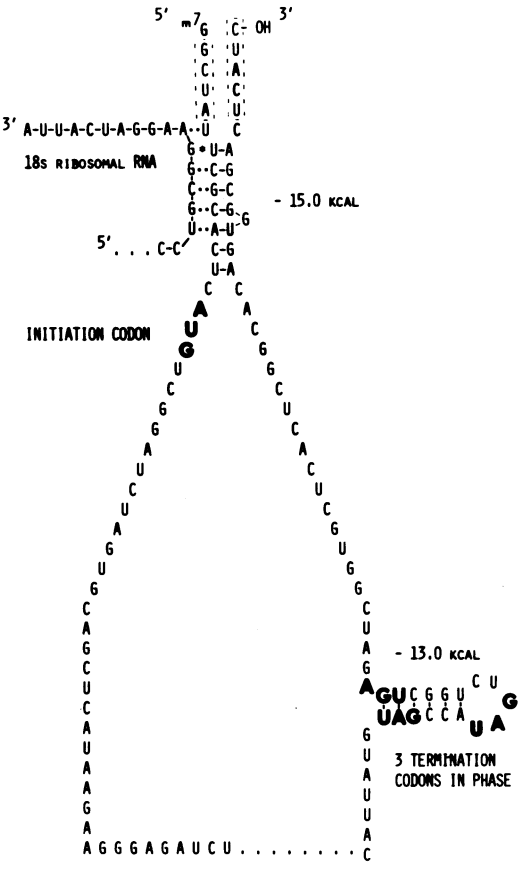


FIG. 6. *Possible partial secondary structure of the s1 mRNA of reovirus serotype 2 (54).*

ture of the controls that regulate frequency of translation; indeed, elucidation of these controls is one of the principal reasons for sequencing the reovirus genes.

## TRANSLATION OF REOVIRUS MESSENGER RIBONUCLEIC ACIDS

The translation of reovirus mRNA's is controlled at several levels. First, there is control mediated by the 5'-terminal cap. The reovirus mRNA's that are transcribed during the first 4 h or so are capped and methylated: they are translated efficiently in extracts of uninfected cells, but not in extracts of cells infected with reovirus (101), whereas uncapped reovirus mRNA molecules are translated poorly in extracts of uninfected cells, but efficiently in extracts of infected cells. Not surprisingly, the immature reovirus particles, which synthesize about 95% of the total viral mRNA that is formed in infected cells (see below), synthesize uncapped mRNA's: their guanylyltransferase and methylase activities are latent (100). It seems, therefore, that some of the factors of the protein-synthesizing mechanism of the cell undergo profound changes during the course of the reovirus multiplication cycle, but we do not yet know what these factors are or how they are modified.

Second, there is profound control over the frequency with which the 10 species of reovirus mRNA are translated, some species being translated much more frequently than others (Table 3). Interestingly, the relative efficiencies of translation seem to be the same both early during the multiplication cycle, when the mRNA's are capped, and late, when they are not capped. It is likely that the major, if not the sole, mechanism for regulating the translation frequencies of these 10 species of mRNA, which exist in the same environment at the same time, resides in their sequence content. Experiments to determine the order of the 10 sequences of mRNA are currently underway via a two-pronged approach involving sequencing the 3' termini of the minus strands of reovirus genes, on the one hand, and cloning reovirus genes and sequencing their DNA transcripts, on the other (Joklik et al., unpublished data). Elucidation of the features of mRNA sequences that specify efficiency of translation could clearly have far-reaching significance. It should be pointed out in this regard that differences in translation frequencies that exist in vivo also exist in vitro: thus, the four *s* species of mRNA are translated in wheat germ protein-synthesizing systems in vitro with efficiencies that differ by as much as sevenfold (53), the same factor that is observed in vivo (Table 3).

The initial events in the translation of reovirus mRNA's have been examined in some detail by Kozak and Shatkin. The first step is the binding of 40S ribosomal subunits at the 5' terminus (41, 44–46). Since there is no binding internally, even

TABLE 3. *Approximate relative frequencies of transcription and translation of the 10 reovirus genes*

| Gene | Transcription frequency | Translation frequency | Translation frequency/transcription frequency |
|---|---|---|---|
| *L1* | 0.05 | 0.03 | 0.6 |
| *L2* | 0.05 | 0.15 | 3 |
| *L3* | 0.05 | 0.1 | 2 |
| *M1* | 0.15 | 0.03 | 0.2 |
| *M2* | 0.3 | 1.0 | 3.3 |
| *M3* | 0.5 | 0.5 | 1 |
| *S1* | 0.5 | 0.05 | 0.1 |
| *S2* | 0.5 | 0.2 | 0.4 |
| *S3* | 1.0 | 0.3 | 0.3 |
| *S4* | 1.0 | 0.7 | 0.7 |

when the RNA is denatured to expose putative masked initiation codons (43), it seems that the binding is specific for 5′ termini, the affinity for capped RNAs being greater than that for uncapped ones which, however, do bind to a significant extent (47). The 40S ribosomal subunits cover a surprisingly large stretch of mRNA, namely, 50 to 60 residues. After they have bound, they move along the RNA molecule until they reach the first initiation codon, where they pause to combine with 60S ribosomal subunits, methionyl transfer mRNA, and whatever factors are necessary to initiate protein synthesis (48). The stretches of RNA covered by 80S ribosomes are shorter than those covered by 40S subunits and are subsets of them; the sequences covered by 80S ribosomes are 25 to 35 residues long and are centered about the initiation codon (41). Pausing at the first AUG is a critical event of the translation process; it depends on secondary RNA structure, since 40S ribosomal subunits do not pause there—and do not combine with 60S ribosomal subunits—if the secondary structure of mRNA is weakened or destroyed (42, 43) or in the presence of inhibitors of protein synthesis such as edeine. It will be fascinating to determine which of these various stages regulates translation frequency (i.e., efficiency).

## ESSENTIAL FEATURES OF THE REOVIRUS MULTIPLICATION CYCLE

Figure 7 shows the essential features of the reovirus multiplication cycle. Reovirus particles adsorb to specific receptors that are the same for all three serotypes (52). The reovirus attachment organ is protein σ1, which, as pointed out above, is located pairwise in 12 icosahedrally distributed positions on the virus particle surface; this protein can adsorb to cells by itself and compete with virus particles for attachment sites. Presumably the initial attachment of reovirus particles to host cells occurs via two σ1 molecules.

The reovirus particle is then taken up into cells in phagocytic vacuoles that fuse with lysosomes (97) within which the reovirus outer capsid shell is partially digested: proteins σ1 and σ3 are removed entirely, and a 12,000-dalton fragment is removed from protein μ1C, leaving a 60,000-dalton protein that is sometimes referred to as protein δ (13, 96, 98). This partial disruption of the outer capsid shell, which generates the so-called SVPs, activates the transcriptase that is present within them, and once the SVP have been released from the lysosomes into the cytoplasm, the reovirus genes begin to be transcribed.

When reovirus genes are transcribed by cores in vitro, in the presence of 5 mM $Mg^{2+}$, they are transcribed with a frequency that is inversely proportional to their size (99) (see above). The frequency of transcription of the 10 genes in vivo differs somewhat from this pattern in that the amount of *s* transcripts usually exceeds the amount of *m* transcripts, which in turn exceeds the amount of *l* transcripts that are formed; this is most probably due to the fact that the intracellular $Mg^{2+}$ concentration is much lower than 5 mM. In addition, it seems that there is some regulation over the rate of transcription of individual reovirus genes during the early stages of the multiplication cycle (113). At that time four species of mRNA are synthesized predominantly: *l*1, *m*3, *s*3, and *s*4. This pattern soon changes to that described above, but persists in the presence of cycloheximide (50) and also oc-

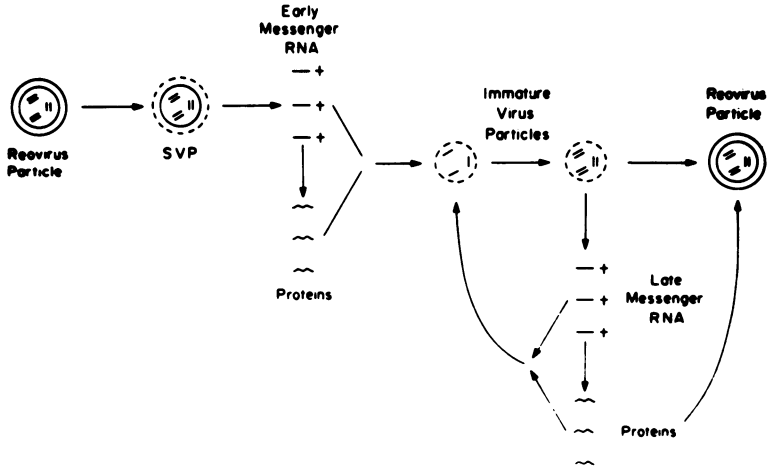


FIG. 7. *Schematic representation of the reovirus multiplication cycle.*

curs under nonpermissive conditions, such as in mammalian cells infected with avian reovirus (104). Furthermore, Shatkin and LaFiandra (90) reported that whereas infectious SVPs (which are produced by digesting reovirus particles with chymotrypsin in vitro in the presence of 0.15 M NaCl and which appear to be identical to the SVPs produced in vivo) transcribe all 10 species of mRNA in vitro, they transcribe only species *l1*, *m3*, *s3*, and *s4* in cells treated with cycloheximide. These data suggest that the transcription of reovirus genes may be regulated early during the multiplication cycle, possibly by a host cell factor the function of which is neutralized by one or more of the proteins coded by genes, *L1*, *M3*, *S3*, and *S4* (namely, the very minor species λ3, the outer capsid shell component σ3 [which has strong affinity for ds RNA], and the two nonstructural proteins μNS and σNS). It is difficult to see, however, how transcription within SVPs can be influenced from the outside when not even pancreatic RNase, a very small protein, can enter them. Clearly, more work remains to be done on the mechanism of this mysterious phenomenon.

The 10 mRNA's are translated into 10 primary translation products. Sizable amounts of these proteins are present in the cytoplasm of infected cells (32). They migrate in density gradients according to their monomeric molecular weights except proteins σ3 and μ1C; about 80% of each of these latter proteins sediments in the form of a complex the molecular composition of which appears to be 1 μ1C:1 σ3. The existence of these complexes is also confirmed by treating infected cell extracts with monoclonal antibodies, when antibodies directed against μ1C and σ3 precipitate not only the free form of each protein but also the complex (51). Interestingly, one antibody precipitates the complex but neither of the free proteins, which indicates that the antigenic site against which this antibody is directed comprises amino acid sequences of both μ1C and σ3.

Several of the primary reovirus translation products are processed to cleavage products. Interestingly enough, in no case is cleavage complete; that is, in all cases some of the primary translation product remains uncleaved, which suggests that both it as well as the cleavage product may have a function during reovirus multiplication. One of these pairs of proteins has already been discussed above: it comprises proteins μ1 and μ1C, the products of gene *M2*. The other two pairs are μNS and μNSC, and λ2 and λ2C (51). These latter two cleavage products were discovered when monoclonal IgG's against μNS and λ2, respectively, were added to extracts of cells infected with reovirus. In the case of μNS, a second protein some 5,000 daltons smaller, which is present in infected cells in roughly the same amount as μNS, was also precipitated, and in the case of λ2, a small amount, corresponding to about 10% of the amount of λ2, of a protein with a molecular weight of about 120,000 was also precipitated. Thus most (μ1), about one-half (μNS), or only a small amount (λ2) of the primary gene product is processed to a cleavage product. The function of μ1C is known (it is the principal component of the reovirus outer capsid shell); that of μNSC and λ2C is not.

## Reovirus Morphogenesis

Some 4 h after infection, presumably when sufficient amounts of viral transcripts and proteins have been formed, morphogenesis commences. The earliest immature virus particles comprise virus-coded proteins among which λ1, λ2, σ2, and μ1C are prominent (64) and contain plus-stranded transcripts in RNase-sensitive form (1). Within these particles, the plus strands are transcribed into minus strands once and once only, and the minus strands remain associated with the plus strands, thereby forming the progeny ds RNA molecules. The nature of the mechanism that specifies that each progeny virus particle receives 1 of each 10 RNA molecules is not known and is one of the principal unsolved problems of reovirology. The reason is that the immature reovirus particles are both multiple in nature and unstable, so that it is difficult to purify them. It has so far been impossible to isolate individual species of such particles and to determine their protein composition, the nature of the RNA that they contain, and the enzyme activities that they possess. All that is known is that (i) assortment of RNA molecules occurs at the stage of ss RNA (1, 84, 85, 122); (ii) the earliest class (or classes) of immature reovirus particles possesses an ss → ds RNA polymerase (a replicase), although it is not clear whether all RNA molecules are transcribed simultaneously (in fact, available evidence suggests that transcription of the *s*, *m*, and *l* class plus strands into minus strands may proceed sequentially [121]); (iii) morphogenesis proceeds through the sequential exchange or addition of virus-coded proteins (124); (iv) immature reovirus particles that already contain ds RNA transcribe it into ss transcripts (mRNA) (65, 83) (in fact, this is where reovirus "multiplies," about 95% of viral mRNA formed in infected cells being synthesized by progeny immature reovirus particles); (v) morphogenesis probably proceeds through a stage of corelike particles (84) (particles resembling cores also accumulate in cells infected with certain classes of temperature-sensitive mutants

at nonpermissive temperatures [64]); and (vi) the final stage of morphogenesis appears to be addition of protein σ3, since if infected cells are pulse-labeled briefly with radioactively labeled amino acids, the virus that can be isolated from them is labeled only in protein σ3 (P. W. K. Lee and W. K. Joklik, unpublished data). In fact, σ3 associates even with parental SVPs, which are thus liberated together with newly formed progeny as particles with an intact inner shell and with an outer shell that consists of proteins σ1, δ, and σ3 (13). It should be noted that the addition of σ3 is the event that triggers the inhibition of the transcriptase and thus causes it to become latent (4) (and, incidentally, may cause it to synthesize oligoadenylates and short transcripts for a brief period of time which would then be sealed into the maturing virus particles; see above).

Thus, the salient feature of the reovirus multiplication cycle is that the assortment of RNA segments into progeny virus particles occurs at the level of ss RNA, which provides the physical and genetic link between parent and progeny virus particles; ds RNA never exists in free, unencapsidated form within infected cells (25). The nature of the replication of ds RNA is thus quite different from that of ds DNA: for ds RNA, its plus and minus strands are synthesized at very different times, and have different fates and different functions. It is also clear that recombinants among reovirus strains are formed at the level of ss RNA, not ds RNA, for it seems that no mechanism exists for "mixing" ds RNA molecules.

Nothing is known concerning the mechanism that specifies that each progeny virus particle is apportioned a set of 10 unique RNA molecules. One approach to investigating this problem is to use antisera to individual reovirus proteins to isolate increasingly complex particles on the morphogenetic pathway. Needless to say, morphogenesis would have to be synchronized for this type of study, but this could be achieved by using temperature-sensitive mutants blocked at the first stage of morphogenesis at nonpermissive temperatures (such as *ts*447) (58) and then shifting down. As for the antibodies, a set of 19 monoclonal IgG's directed against 7 of the 10 reovirus-coded proteins has recently been isolated and characterized (51) and could be used for this purpose.

### Reovirus Deletion Mutants

Although the mechanism that apportions RNA molecules to nascent reovirus progency particles is efficient (since most reovirus particles contain 10 genes), it is not infallible, and several examples of reovirus particles with less than 10 genes are known. For example, repeated cultivation of the Dearing strain of reovirus serotype 3 under conditions of high multiplicity results, within seven to eight passages, in the production of noninfectious virus particles that lack gene *L*1 (71, 87). Several temperature-sensitive mutants of reovirus lose genes even more rapidly, the most extreme example being mutant *ts*447, a mutant totally (that is, more than 99%) defective in the ability to form ds RNA-synthesizing particles (i.e., the earliest immature virus particles) at nonpermissive temperatures, yields of which comprise even at permissive temperatures equal numbers of virus particles that contain and lack gene *L*3 (87). When this mutant is cultivated repeatedly at high multiplicity, it yields, within four passages, particles that lack genes *L*1, *L*3, and *M*1 (2, 87). Virus particles lacking genes are also associated with the chronic (persistent) intracerebral infections that result in about 50% of 2-day-old rats inoculated subcutaneously with wild-type reovirus or some of its temperature-sensitive mutants (103).

## TEMPERATURE-SENSITIVE MUTANTS OF REOVIRUS

Fields and Joklik (17) isolated a series of temperature-sensitive mutants that fell into six complementation and gene reassortment groups. During the next several years, the lesions in many of these mutants were assigned to specific genes by the use of both biochemical and genetic techniques; the four remaining mutant groups were isolated by Ramig and Fields in 1979 (74). Their genetic and phenotypic properties are summarized in Table 4.

Reovirus exhibits the phenomenon of extragenic suppression to a remarkable extent. In 1977, Ramig et al. (76) found that a spontaneous revertant of a temperature-sensitive mutant of

TABLE 4. *Reovirus temperature-sensitive mutants*[a]

| Reassortment group | Type | Lesion in gene[b] |
|---|---|---|
| A | Late | L2 (86) or M2 (66) |
| B | Late | L2 (66) |
| C | Early | S2 (58, 75) |
| D | Early | M2 (35) or L1 (75) |
| E | Very early | S3 (34, 75) |
| F | Late | ? |
| G | Late | S4 (66) |
| H | ? | ? |
| I | ? | ? |
| J | ? | ? |

[a] Mutant groups were isolated as follows: A to E, by Fields and Joklik (17), F and G by Cross and Fields (14); and H to J by Ramig and Fields (74).

[b] References providing evidence concerning the locations of the lesions are given in parentheses.

reovirus (the group A mutant *ts*201) did not result from back mutation to wild type, but rather from the presence of a second mutation (a "suppressor" mutation) elsewhere in its genome. This was shown by backcrossing the revertant to wild-type virus and demonstrating that about one-half of the progency still harbored the original temperature-sensitive lesion; the backcross served to separate the temperature-sensitive from the suppressor mutation. This phenomenon of extragenic suppression is very common in reovirus. Thus Ramig and Fields (74) found that 25 of 28 spontaneous revertants of a variety of temperature-sensitive mutants belonging to all mutant groups still contained temperature-sensitive lesions, and they were even able to isolate new suppressed temperature-sensitive lesions belonging to new mutant groups from the pseudorevertants, which may have acted as temperature-sensitive suppressors. The significance of this work stems from the fact that the suppressor mutations probably act by producing compensating alterations in some other protein that interacts physically with the protein coded by the gene with the temperature-sensitive lesion, and one would therefore expect the existence of precise pairwise patterns of temperature-sensitive suppressor lesions. Their elucidation should reveal the existence of interactions among reovirus-coded proteins that may provide important clues for reovirus morphogenesis.

## INTERACTION OF REOVIRUS WITH THE INTACT HOST

As pointed out above, reovirus was originally isolated from two distinct sources: the human gastrointestinal tract and the brains of mice in which it had produced runting disease. Fields and his collaborators have recently attempted to define which genes of reovirus are responsible for virulence and tissue tropism. They have used for this purpose strains of the three serotypes of mammalian reovirus, as well as some of the temperature-sensitive mutants in the collection of Fields and Joklik. They have found that at least two genes govern the interaction of reovirus with its host. First protein $\sigma$1, coded by gene *S*1, is crucial for specifying the interaction between reovirus and its host. It is the reovirus cell attachment protein (52) and to that extent specifies whether reovirus particles can or cannot interact with particular types of cells; furthermore, the fact that it interferes, in its free native form, with the adsorption of virus particles of all three serotypes of reovirus demonstrates that it interacts with specific receptors on cell surfaces. It is also the reovirus hemagglutinin (119) and is one of three reovirus proteins that gives rise to neutralizing antibody (the others being $\lambda$2 and $\sigma$3) (31, 116). It also controls the intracellular association of reovirus particles with microtubules (5). Furthermore, it is responsible for the generation of cytolytic T lymphocytes after infection, the development of delayed-type hypersensitivity (117) and the generation of suppressor T cells (19, 29). It also controls tissue tropism to the extent that it determines neurovirulence patterns (115, 118): intracerebral inoculation of reovirus serotype 3 into newborn mice causes a necrotizing encephalitis that is uniformly fatal, whereas reovirus serotype 1 causes ependymal cell damage and hydrocephalus, but the animals generally survive. By using recombinants of reovirus serotypes 1 and 3, it is readily shown that the neurovirulence pattern is associated solely with the nature of the *S*1 gene that they contain, and is presumably predicated by the interaction of $\sigma$1 with receptors on neuronal/ependymal cells.

Protein $\mu$1C also controls virulence and tissue tropism, but by a totally different mechanism. That it can be shown to do so is a striking illustration of the often suspected but difficult to demonstrate principle that virulence is an extremely complex phenomenon mediated by numerous processes and pathways. Protein $\mu$1C, it will be recalled, is the major constituent of the reovirus outer capsid shell which is removed when treatment with proteases such as chymotrypsin converts reovirus particles to cores, which are far less infectious. It appears that the $\mu$1C of serotype 1 is much more resistant to proteases than that of serotype 3 (81). As a result, when it reaches the intestinal tract, reovirus serotype 1 is resistant to the proteases that it then encounters and is able to cause infection in intestinal tissue, whereas serotype 3 strains are inactivated and fail to initiate significant infections. Interestingly, when a recombinant is inoculated into the upper intestinal region that possesses an *M*2 gene of serotype 1 and an *S*1 gene of serotype 3, fatal intracerebral infection results from initial multiplication in intestinal tissue and subsequent spread to the brain (see above). Gene *M*2 thus represents a second reovirus virulence gene, for diseases or dietary factors that reduce the secretion or activity of gastric or pancreatic enzymes could clearly increase susceptibility to reovirus through the mediation of the protein that it encodes.

This type of study, the use of recombinants in a system in which gene reassortment both occurs readily and is readily detected, is an excellent example of the application of basic research to clinical problems, which in the final analysis is the major goal of virologists, be they basic or clinical.

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